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Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 265, No. 35, Issue of December 15, pp. 21430-21432, 1990

Singl Crystals of a Chimeric T7/T3 RNA Polymerase with T3 Promoter Specificity and a Nonprocessive T7 RNAP Mutant*

(Received for publication, June 14, 1990)

Rui Sousa‡§, Yong Je Chung¶, William T. McAllister‖, B.-C. Wang‡¶, and Eileen M. Lafer‡

From the ‡Department of Biological Sciences and the ¶Department of Crystallography, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 and the ‖Department of Microbiology and Immunology, State University of New York Health Science Center, Brooklyn, New York 11203-2098

Two RNA polymerases homologous to bacteriophage T7 RNA polymerase, bacteriophage Sp6 and T3 RNA polymerases, were screened for crystallization under conditions identical or similar to those reported for the growth of large single crystals of T7 RNA polymerase (Sousa, R., Rose, J. P., Chung, Y. J., Lafer, E. M., and Wang, B.-C. (1989) *Proteins* 5, 266; Sousa, R., Lafer, E. M., and Wang, B.-C. (1990) *J. Crystal Growth*, in press; Sousa, R., and Lafer, E. M. (1990) *Methods* 1, in press). A number of mutant T7 RNAPs were also screened under these conditions as were three chimeric RNA polymerases consisting of T7 RNAP N-terminal and T3 RNAP C-terminal sequences. One chimeric polymerase and two mutant polymerases crystallized readily under T7 RNAP crystallization conditions. The chimeric polymerase crystallized in a space group different from T7 RNA polymerase: orthorombic with unit cell parameters $a = 75$ Å, $b = 98$ Å, $c = 159$ Å; space group $P2_12_12_1$ and 4 molecules/unit cell. This chimeric enzyme exhibits T3 promoter specificity and will make it possible to investigate how structural differences between the T3 and T7 RNA polymerase promoter recognition domains determine their different promoter specificities. One of the mutant polymerases successfully crystallized was an enzyme which can carry out promoter recognition and abortive transcription but cannot carry out processive transcription. Its structure may provide information on the nature of the conformational changes undergone by T7 RNAP in the abortive-processive switch. Crystals of the second mutant T7 RNA polymerase were unsuitable for x-ray analysis.

Bacteriophage T7 RNA polymerase is a member of a family of relatively small, monomeric RNA polymerases which includes the bacteriophage Sp6 and T3 RNA polymerases (4-6), the yeast mitochondrial RNA polymerase (7) and probably other eukaryotic mitochondrial RNA polymerases (8). We have previously reported the preparation of large single crystals of T7 RNA polymerase suitable for x-ray analysis (1-3). The crystallization of this enzyme prompted us to attempt to crystallize the homologous Sp6 RNA polymerase and the highly homologous T3 RNA polymerase under the conditions defined for crystallization of T7 RNA polymerase. Since the primary functional difference between the T7, Sp6, and T3 RNA polymerases is that they recognize different promoter sequences, the overiding motive in seeking to crystallize these enzymes was to allow questions of promoter specificity to be addressed by docking studies and structural comparison. We also examined crystallization of a number of chimeric T7/T3 RNA polymerases (9). Because T7/T3 gene 1 crosses had previously indicated that the C-terminal portions of the T7 and T3 RNAPs were critical for differentiating between the T7 and T3 phage promoters (10), Joho *et al.* (9) constructed a number of recombinant T7/T3 RNAP[1] genes in which the N-terminal two-thirds to three-fourths of the gene consisted of T7 sequences, while the C-terminal portion consisted of T3 sequences. The resultant chimeras displayed T3 promoter specificity but were otherwise fully functional RNA polymerases with no other significant functional or biochemical differences between the chimeras and the wild type T7 RNAP having been noted (9). Crystallization of a number of other mutant T7 RNA polymerases whose x-ray analysis could provide useful information or facilitate the ongoing structure determination of the wild type T7 RNAP, was also attempted. In particular we were interested in obtaining crystals of mutant polymerases whose *in vitro* phenotypes were indicative of effects on the conformational changes undergone by the polymerase during transcription.

MATERIALS AND METHODS

Purification and Crystallization—RNA polymerases were purified as described previously (1, 2). For crystallization, polymerases were dialyzed into 40% saturated ammonium phosphate (SAP) pH 7.0, 8.0, or 9.0, 24% glycerol, 1 mM EDTA, 1 mM dithiothreitol and concentrated by centrifugation using Amicon centricon tubes to concentrations of 20-50 mg/ml (30 mg/ml was most typically used). 100% SAP was prepared by dissolving dibasic ammonium phosphate in warm water until saturation was reached; ammonium hydroxide and phosphoric acid were used to obtain the desired pH and the solution was then capped and placed at 4 °C for several days before being filtered for use in setups. The addition of GTP and $MgCl_2$ was examined by addition of GTP or $MgCl_2$ to a final concentration of 10 mM (GTP), or 2 mM ($MgCl_2$) to the protein sample immediately prior to setting up for crystallization. The protein in 40% SAP was equilibrated with a reservoir of 45% SAP, 27% glycerol, or 50% SAP, 30% glycerol by vapor diffusion of sitting drops of 10-50-μl volumes. After allowing 1 week for equilibration, duplicate setups were left unseeded or were seeded with a suspension of T7 RNAP microcrystals. Activity assays and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of polymerase from dissolved crystal were carried out as described previously (1).

X-ray Data Collection and Analysis—X-ray analysis was carried out as described previously (1).

* This work was supported by National Institutes of Health Grant GM 41936 (to B.-C. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Dept. of Biological Sciences, A524 Langley Hall, Pittsburgh, PA 15260. Tel.: 412-624-4826.

[1] The abbreviations used are: RNAP, RNA polymerase; SAP, saturated ammonium phosphate.

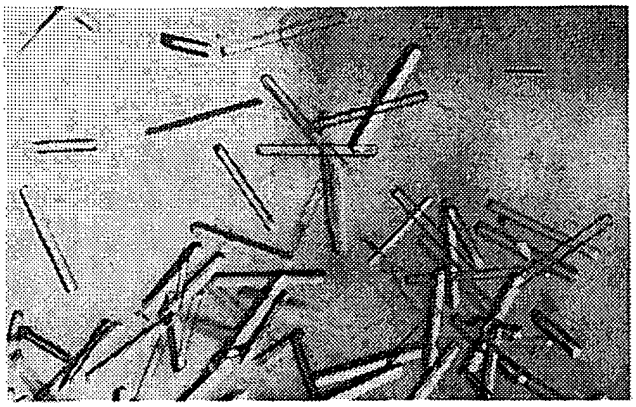

FIG. 1. **Orthorombic crystals of the chimeric T7/T3 RNA polymerase expressed from construct pLG2.** The *bar* is 0.4 mm in length.

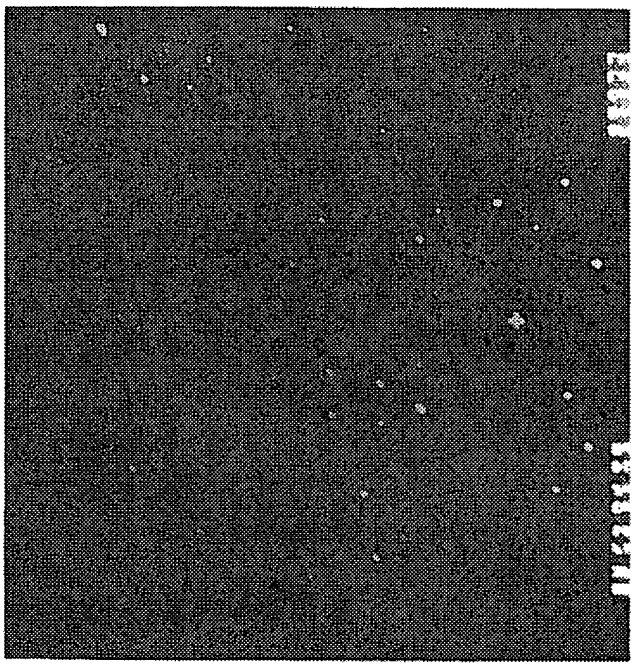

FIG. 2. **Diffraction pattern from a crystal of pLG2 chimeric T7/T3 RNA polymerase as recorded on a Nicolet area detector (300-s exposure, 0.25° oscillation).** The *cross* indicates the direct beam position (D = 16 cm, $\phi = 12°$, the detector face is 10 cm across, resolution at the left edge is 3.2 Å). Diffraction to 3.5 Å is visible.

RESULTS

Purification and Crystallization—RNA polymerases were purified as previously described for preparation of T7 RNA polymerase (1, 2). Briefly, this procedure utilizes ammonium sulfate and polymin P fractionation followed by ion exchange chromatography on Whatman phosphocellulose, DEAE-Sepharose (Pharmacia LKB Biotechnology, Inc.), and sizing on Sephacryl S-200 (Pharmacia). Details of the crystallization procedure are given under "Materials and Methods."

Preliminary X-ray Analysis of the Crystals of Chimeric T7/T3 RNAP (pLG2)—The crystals grown from the p3234 polymerase (addition of Phe-Ala to the C terminus, wild type activity) were not suitable for x-ray analysis. The crystals of p3214 polymerase were isomorphous with the T7 RNAP crystals. These crystal parameters have been reported previously (1). Diffraction data on the T7/T3 chimeric crystals was recorded on a Nicolet X100 multiwire area detector using CuKα x-rays (5 kilowatts). Fresh crystals (1.2 × 0.15 × 0.15 mm, Fig. 1) diffracted to 3.5 Å (Fig. 2). Analysis of the diffraction data revealed that these crystals belonged to the orthorombic space group $P2_12_12_1$ with a = 75 Å, b = 98 Å, and c = 159 Å and contained 1 molecule/asymmetric unit. The resultant solvent content is therefore estimated to be 58%.

DISCUSSION

Of a number of homologous (Sp6, T3), mutant (p3234, HK20, p3214, p3225, pAR3153, pKM786), and chimeric (pNM44, pKJ33, pLG2) RNA polymerases tested, we have obtained crystals of three enzymes under conditions similar to those previously identified as suitable for crystallization of T7 RNAP (1–3). Only two of these (p3214, pLG2) gave crystals suitable for x-ray analysis, one of which (p3214) was isomorphous with the previously described crystals of T7 RNAP. The chimeric polymerase expressed from pLG2 consists of T7 residues 1–673, and T3 residues 674–884. Within these 200 C-terminal residues there are 26 amino acid substitutions between the T3 and T7 sequences. Six of these substitutions cluster in residues 740–750. The polymerase expressed from p3214 contains a deletion of its two most C-terminal residues, and has been shown to be capable of carrying out promoter-dependent abortive transcription but to be unable to engage in fully processive transcription.[2] The structure of this mutant may therefore be revealing of the conformational changes proposed to be undergone by T7 RNA polymerase in the switch from abortive to processive transcription (11, 12).

Recombinant DNA techniques have found wide and varied application in crystallographic studies of macromolecular structure. The crystallization of the T7/T3 chimera described here represents a modest addition to this variety of application. In its broadest sense this concept involves swapping domains responsible for substrate/ligand specificity from a noncrystallizable protein onto a homologous, crystallizable protein in the hopes that the resultant hybrid molecule will crystallize and allow comparative studies of the structures of the substrate/ligand specificity determining domains. It is not clear to what degree this concept is supported by the results of these experiments; *i.e.* the chimeric polymerase crystallized in a space group different from T7 RNAP casting doubt on the supposition that its crystallization depended on a duplication of the crystal packing contacts found in the T7 RNAP crystal. Such questions will be answered by the ongoing x-ray studies: it is possible that, despite the differences in space group, similar crystal packing contacts are utilized in both crystals. In particular, noncrystallographic pseudo-symmetries in the trimeric asymmetric unit of the monoclinic T7 RNAP crystals may be utilized as crystallographic symmetries in the orthorombic, chimeric crystals. Such relationships have been observed in other systems (*i.e.* it has been inferred that pseudo-symmetry elements in the *Eco*RI endonuclease crystal are related to crystallographic symmetry elements in the *Eco*RI endonuclease-DNA co-crystal (13)). Relative to the monoclinic T7 RNAP crystal this orthorombic crystal offers the potential advantages of reduced unit cell volume, reduced asymmetric unit size, and increased space group symmetry (reduced data collection time for equivalent data redundancy). While the crystals prepared to date diffract to only 3.5 Å at best, these crystals are still small and it is expected that larger crystals diffracting to higher resolution will be grown. Relative to crystals of T7 RNA polymerase of equal size, the chimeric

[2] J. Coleman, unpublished observations.

crystals diffract more strongly. In the near team, x-ray analysis of the chimeric polymerase crystal may allow us to unambiguously assign domains in the T7 RNAP structure through rotation and difference methods and may facilitate chain tracing in the C-terminal portion of the polymerase. Ultimately, it will allow us insights into the mechanism of promoter recognition in this family of polymerases by allowing a comparative study of the structures of the promoter recognition domains of the T7 and T3 RNA polymerases.

Acknowledgments—We thank Dr. Joseph Coleman for his gift of plasmid pKM789. We thank Drs. John Dunn and William F. Studier for their gift of plasmids p3234, p3214, p3225, and pAR3153.

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